

Characterisation of The Regenerative Ability of The MRL/MPJ Mouse in Order to Re-Innervate and Promote Scar-Free Healing in Engineered Skin Equivalents

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INTRODUCTION: Understanding morphogenesis of tissues is key to advancing cell and tissue based therapies collectively known as regenerative medicine. We have identified a mutant mouse model that may help us understand mammalian scar free skin regeneration. The MRL/MpJ mouse displays an unusual trait amongst mammals in that it has the ability to perfectly regenerate skin and cartilaginous components without scar tissue formation. Following tissue loss due to an ear punch wound, histological analysis has revealed the formation of a blastema-like structure 14 days post-wounding, showing many parallels with regenerating amphibian limbs and mammalian limb embryogenesis.¹ We aim to identify the differential expression of candidate regenerative genes and their translated protein products responsible for the formation of a blastema-like structure and regeneration of peripheral nerves in the MRL/MpJ model.

METHODS: Mice wounded with a 2mm biopsy punch to the centre of each ear and a 4mm biopsy punch to the back, were harvested up to 112 days post-wounding. Immunohistochemical co-localisation with antibodies directed against PAN-Neurofilament (NF) and the endothelial marker CD31 was used to visualise both nerves and blood vessels at the wound site. To detect differential expression of key genes, RNA probes were created for *in situ* hybridisation to sections of regenerating ears. Candidate growth factors and developmental markers such as FGF8, FGF10, Hoxb13, Hoxa9, Hoxa13 and Msx1 may potentially regulate blastema formation within the mouse ear, and this is currently under investigation.

RESULTS: Immunohistochemical analysis has shown the murine ear to be highly innervated, but more interestingly, from 10 days post wounding, the MRL/MpJ mouse exhibits a greater capacity for peripheral nerve regeneration compared to the poorer healing C57BL/6 and BALB/c strains. Co-localisation of CD31 with PAN-Neurofilament has revealed that the pattern of re-vascularisation within the blastema-like structure is closely associated with the regenerating nerve network. Furthermore, nerve regeneration into the blastema precedes blood vessel growth (fig 1).

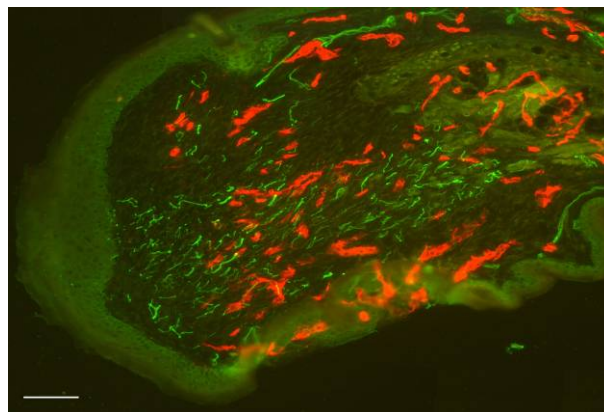


Fig. 1: MRL/MpJ ear blastema 21 days post wounding. CD31 (red), NF (green) (bar = 100µm).

However, in the same animal, an excisional back wound undergoes repair and subsequent scar formation.² Unlike the ear wound blastema, we have shown that blood vessels are the first to infiltrate the back wound area and nerve regeneration follows.

DISCUSSION & CONCLUSIONS: Initial results indicate that during regeneration in the ear, the nerve network appears to act as a template for angiogenesis, recapitulating the process of early development. The accelerated nerve regeneration observed in the MRL/MpJ mouse may contribute to its regenerative capacity. The molecular mechanisms involved in this trait are yet to be determined. Further investigations will ascertain if other differentiated structures, such as hair follicles, arise within the blastema region *de novo*. The co-localisation of Schwann cells and regenerating nerves during blastema formation is also underway. The ultimate goal is to harness the molecular signalling pathways responsible for the generation of the blastema-like structure and subsequent nerve regeneration for use within an engineered skin substitute.

REFERENCES: ¹ C. Rajnoch, *et al.* (2003) *Developmental Dynamics*, **226** (2): 388-97, ² Metcalfe, A.D & M.W.J Ferguson (2005) *Biochemical Society Transactions* **33**: 413-417.

ACKNOWLEDGEMENTS: This research has been supported by grants from the MRC, BBSRC and EPSRC.